

***Rana iberica* (Boulenger, 1879) goes underground: subterranean habitat usage and new insights on natural history**

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Abstract

Reports of amphibians exploiting subterranean habitats are common, with salamanders being the most frequent and studied inhabitants. Anurans can occasionally be observed in caves and other subterranean habitats, but in contrast to salamanders, breeding had never been reported in a cave or similar subterranean habitat in Western Europe. Based on observations during visits to a drainage gallery in Serra da Estrela, Portugal, from May 2010 to December 2012, here we document: (i) first report of *Rana iberica* reproduction in cave-like habitat, representing the fourth report of an anuran for the Palearctic ecozone; (ii) oophagous habits of the tadpoles of *R. iberica*; and (iii) *Salamandra salamandra* predation on *R. iberica* larvae. These observations, particularly of *R. iberica*, highlight our lack of knowledge of subterranean ecosystems in the Iberian Peninsula.

Keywords

Subterranean habitats, amphibians, anuran reproduction, larval predation, oophagy

Introduction

Caves and other subterranean habitats contain a biodiversity that has long intrigued biologists, such as Darwin and Lamarck (Culver and Pipan 2009). Often limited in light, these places have revealed a great number of diverse and unique species (Romero 2009). The occurrence of amphibians in caves and associated subterranean habitats is not novel (e.g. Manenti 2008). Amphibians can use caves and other subterranean habitats for thermal refugia (Uhrin and Lesinsky 1997, Fenolio et al. 2005), to avoid predation (Manenti et al. 2009), as feeding habitats (Baumgart 1981, Resetaritis 1986, Uhrin and Lesinsky 1997) and as breeding sites (Diesel et al. 1995). A number of salamanders are troglobitic (obligate cavernicole species unable to survive outside low-light environments; Hoffmann 2002) restricted to cave habitats and exhibiting troglomorphic characters, such as degenerate eyes and depigmentation. Other amphibians use caves to complete some aspect of their life cycles and may or may not exhibit some level of troglomorphy. Anurans are occasionally observed in subterranean habitats, but no species are known to be obligate cavernicoles (Culver and Pipan 2009; Romero 2009).

In the Palearctic region, amphibians are relatively common in subterranean habitats; however only one species is obligate cavernicola (*Proteus anguinus* Laurenti, 1768). Other salamanders are known to use caves during part of their life cycles (e.g., *Paradactylodon persicus* (Eiselt & Steiner, 1970), *Speleomantes* spp. Dubois, 1984, *Chioglossa lusitanica* Bocage, 1864 and *Salamandra salamandra* (Linnaeus, 1758) (Sequeira et al. 2001, Kami 2004, Lanza et al. 2006, Manenti et al. 2009, Romero 2009, Ianc et al. 2012). Reports of anurans are frequent in Palearctic subterranean habitats (e.g., McClure et al. 1967, Baumgart 1981, Bonini et al. 1999, Bressi and Dolce 1999, Presetnik et al. 2002, Biswas and Shrotriya 2011), however breeding reports and limited to few species. During a regular monitoring of the amphibian community at Portofino Park (Liguria, north-western Italy) in 1996, Bonini et al. (1999) found tadpoles of *Bufo bufo* (Linnaeus, 1758) inside an artificial tunnel with a part of complete darkness, and kept on finding egg strings in subsequent years. These were then the first evidences of anuran reproduction inside subterranean habitat in this ecozone. In 1998, Gasparo and Stoch observed eggs of *Rana temporaria* Linnaeus, 1758 inside a natural cave (Kompoljska jama) in Dobropolje (Slovenia) (Bressi and Dolce 1999). Additionally, in a visit to the Viršnica - Jamski cave system in Radenskem polju (Central Slovenia), Presetnik et al. (2002) discovered four egg-clutches and several adults of *Rana dalmatina* Bonaparte, 1840. Frogs were found more than 500m from the giant tunnel entrance, but as in the previous record, data on biology, phenology and cave habitat usage is also still largely missing.

Worldwide, breeding by anurans in subterranean habitats has only been confirmed for three additional species of leptodactylid frogs in the Neotropic ecozone: two of genus *Eleutherodactylus* Duméril and Bibron, 1841 inhabiting Caribbean islands (Diesel et al. 1995, Rogowitz et al. 2001) and *Cycloramphus eleutherodactylus* (Miranda-Ribeiro, 1920) from Brazil (Lima et al. 2012).

The Iberian brown frog (*Rana iberica* Boulenger 1879) is endemic to Portugal and north-western and central Spain. Although it is a typical mountain species, this frog can be common in coastal areas at low altitude. The species inhabits streams with cold and clear waters, rocky substrates, and abundant vegetation surrounding the margins (Loureiro et al. 2008). *Rana iberica* can also be found in other habitats, including small ponds, humid meadows and soaked fields. The breeding season occurs from November to March in the lowlands and from March to May in the highlands (Crespo and Cei 1971, Almeida et al. 2001, Salvador and García-París 2001, Ayres 2008). Females lay small clutches of eggs (between 100-450) with a gelatinous coat, beneath underwater stones or stuck to water-weed (Almeida et al. 2001, Ayres 2009). No records of this species inhabiting subterranean habitats are known.

Predators of *R. iberica* have been documented across all life stages. Adults are prey of water snakes (*Natrix maura* (Linnaeus, 1758) and *N. natrix* (Linnaeus, 1758)), *Vipera seoanei* Lataste, 1879, small carnivores such as *Neovison vison* (Schreber, 1777), *Genetta genetta* (Linnaeus, 1758) and *Lutra lutra* (Linnaeus, 1758) (Galán-Regalado 1988, Almeida et al. 2001; Morales et al. 2004), and even the barn owl, *Tyto alba* (Scopoli, 1769) (Rey et al. 2004). Larvae are vulnerable to several aquatic insects, trout, water snakes, and even the marbled newt, *Triturus marmoratus* (Latreille, 1800) (Almeida et al. 2001, Bosch et al. 2006). There are no records of predation by other salamander species to our knowledge.

Here, we report for the first time evidence of *Rana iberica* reproduction in a subterranean habitat, the fourth record of an anuran for the Palearctic ecozone. Furthermore, we discuss the natural history of *R. iberica*, including the report of a new predator of larvae and oophagous habits of its tadpoles.

Methods

Serra da Estrela Natural Park is located in north-central Portugal (40°20'N, 7°35'W) and is the largest protected area with approximately 89000 hectares (Fig. 1). It is one of the highest mountains (1993 m a.s.l. at Torre Plateau) in the Iberian Sistema Central. The climate is temperate-Mediterranean with Atlantic influences, presenting dry and warm summers and a wet season from October to May with frequent snowfall at higher altitudes (Daveau et al. 1977, Andrade et al. 1992, Jansen and Sequeira 1999). Rich in freshwater environments, Serra da Estrela comprises many permanent ponds and lakes of glacial origin, swamps, bogs, temporary pools, and a dense system of springs, brooks and rivers (Costa et al. 2004). The mean annual temperature is 3-4°C at the highest altitudes (Vieira and Mora 1998) and mean annual rainfall is greatest at the Planalto Superior (exceeding 2500 mm) and least in the northwest region (about 900 to 1000 mm) (Daveau et al. 1977, Jansen 2002).

Because of its geographic location, topographic and climatic conditions, Serra da Estrela has several ecosystems that are unique in Portugal. In addition, this area con-

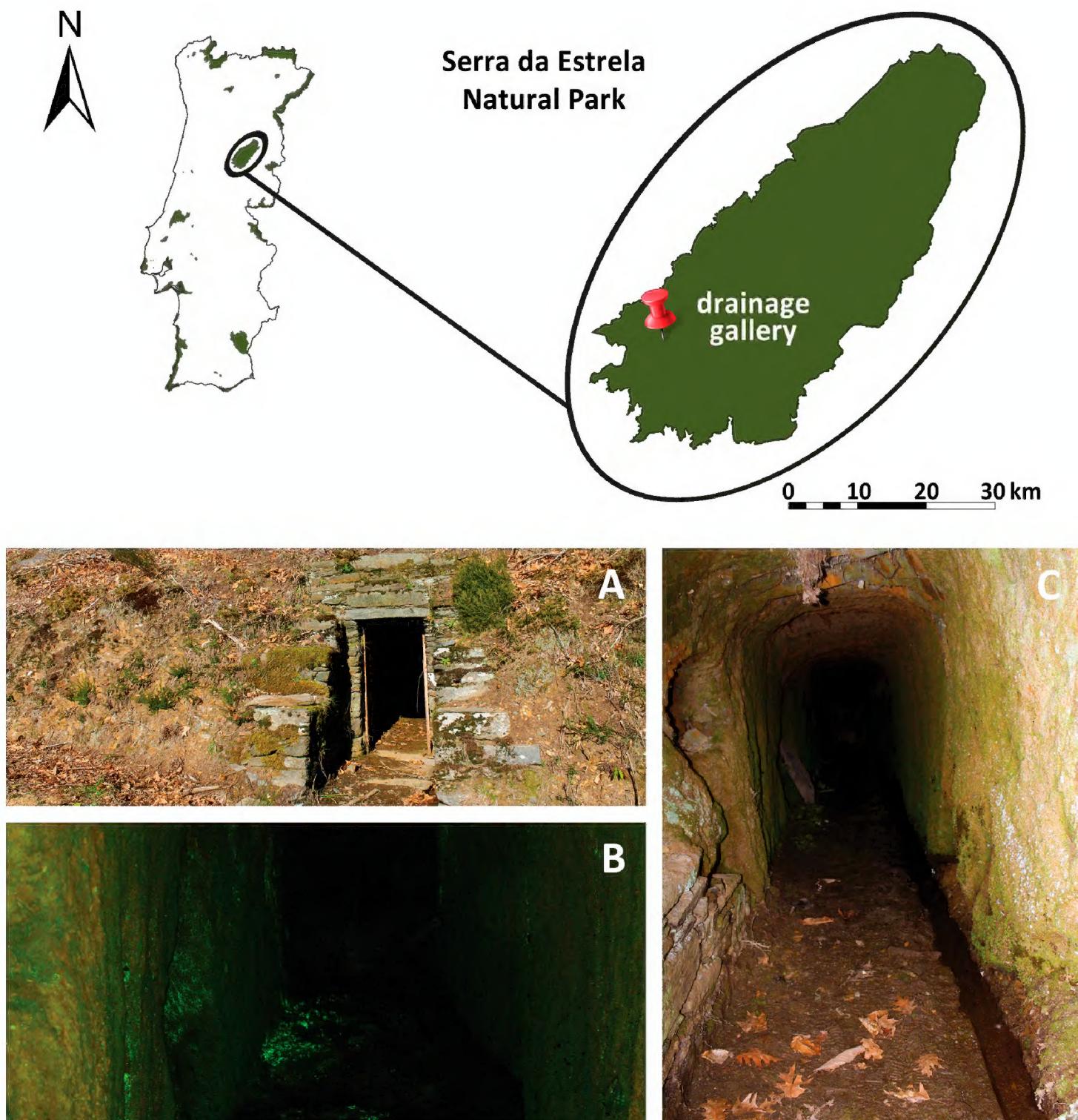


Figure 1. Serra da Estrela Natural Park and hypogean habitat used by individuals of *Rana iberica*: **A** entrance of the underground spring **B** *Schistostega pennata* covering walls and floor of the drainage gallery **C** horizontal tunnel of drainage gallery. Photo A by Madeira M, B by Rosa GM, C by Laurentino T.

tains many rare and endemic species. This region is one of the most biodiverse in Portugal and is considered an Iberian Peninsula biodiversity hotspot (Teles 1970, Costa et al. 1998, Moreira et al. 1999, Costa et al. 2003). Among amphibians, Serra da Estrela harbors 13 species, including five species of salamanders and eight species of anurans (Brito-e-Abreu et al. 1994, Lesparre and Crespo 2008).

Several drainage galleries were created for water capture in the 1950s, even before the establishment of the boundaries of the Natural Park. Galleries are characterized by a horizontal tunnel that penetrates the side of a hill. Typically, galleries are of limited width (ca. 1 m) and height (1.5 m), and extending several meters into the hillside (Fig.

1A–C; see also Penado et al. in press). These tunnels are cool and humid and often contain a small stream. These artificial subterranean habitats are often used as a refuge for many species, and are particularly important for *Chioglossa lusitanica*, an Iberian endemic of conservation concern (Cabral et al. 2005, ICNB 2006).

During an initial visit to one drainage gallery ($40^{\circ}20'36.95''\text{N}$, $7^{\circ}42'50.23''\text{W}$, 985 m a.s.l.; Fig. 1) near Sazes da Beira (Seia) gallery in May 2010, several (not quantified) *R. iberica* were observed within this subterranean habitat. This particular gallery extended 25 m with walls, as well as some patches of the floor, partially covered by goblin's gold (*Schistostega pennata* (Hedw.) Web. & Mohr) (Fig. 1B). It has a door that is partially closed (with no light penetrating beyond about 5 m, when the door is opened), providing a true dark zone to this habitat. In addition, a small, shallow stream (average of 5 cm water depth) with low flow ran the length of the gallery. Water pH of 7.1 measured on 24 April 2011.

The unusual sighting of *R. iberica* motivated a series of subsequent visits that started in 2011: every three months until November 2011 and monthly between December 2011 and December 2012 to understand the use of this artificial subterranean habitat by this species. We obtained data on the activity cycle and reproductive period of *R. iberica* by noting first the presence of individuals inside the gallery, secondary sexual characters (nuptial pads in males) and breeding behaviour (individuals in amplexus), and the presence and developmental stage of larvae. Based on previous data (Crespo and Cei 1971, Brito-e-Abreu et al. 1994, Lesparre and Crespo 2008), we also generated polar coordinates, allowing a qualitative comparison between the activity of the population inside the gallery and the population of the Planalto Superior region (1400–1993 m a.s.l.).

Results and discussion

Phenology and subterranean biology of *Rana iberica*

All life stages were observed in the gallery during the study period, particularly adults, which were observed every month of the year (Table 1; Fig. 2–4). Adults were observed active both during the day and night often standing on the ground or in crevices (Fig. 3B), swimming underwater or floating, seeking refuge in holes or even climbing up the walls (Fig. 3C) (inactivity being defined by lethargy, habitually observed in hibernating frogs outside the gallery, found hidden under rocks during winter months). Individuals were mainly found beyond the first 5 m from the entrance, where the most intense daylight penetrates. These individuals often presented the lichen-shaped spots on their backs that are typical of this region (Fig. 3A; Malkmus 2004).

In both years of this study, the breeding season occurred between December and April (Fig. 2). Unfortunately, the little available data on the activity of *R. iberica* in Serra da Estrela is confined to the higher elevations (Planalto Superior, area above 1400 m and up to the summit). Although the gallery studied is located about 400 m below

Table 1. Absolute number of *Rana iberica* individuals (post-metamorphics or larvae) and clutches of eggs found per visit (month) inside a drainage gallery at Serra da Estrela Natural Park, Portugal. In some months data is restricted to *detected* (+) and *not detected* (-).

Year	Month	Post-metamorphics			Larvae	Clutches of eggs
		Males	Females	Juveniles		
2010	May	+	+	+	+	+
2011	May	+	+	+	+	+
	August	9	6	2	1	0
	November	3	1	1	0	0
	December	+	+	-	-	-
2012	January	7	6	0	31	6
	February	+	+	+	+	+
	March	6	4	0	64	4
	April	8	4	0	52	2
	May	7	3	4	71	1
	June	6	4	6	12	0
	July	+	+	+	+	-
	August	8	5	8	7	0
	September	5	3	1	2	0
	October	+	+	+	-	-
	November	1	0	1	0	0
	December	+	+	+	-	-

this limit, we noticed obvious differences in the activity patterns of these populations (Fig. 2). Because of very low temperatures, individuals seem to hibernate or reduce activity between December and February in the Planalto Superior population. Additionally, Crespo (1974) show in controlled experiments, that between 2–5°C there is no spermatogenic activity, which must be a reflex of what is happening in the natural habitat in these cool months. The breeding season usually begins in March (Spring) (Brito-e-Abreu et al. 1994, Lesparre and Crespo 2008); Lesparre and Crespo (2008) reported that *R. iberica* is active year-round in low elevation populations of the Serra da Estrela. However the altitudinal limit of year-round activity is unknown and no data are available on the breeding period. Nonetheless, the gallery population in the present study shows a pattern overall similar to other *R. iberica* populations occurring at lower elevation, described by Galán-Regalado (1982). Year-round activity of the gallery population may be related to the stability in environmental conditions occurring in this artificial subterranean habitat, which includes less variation in air temperatures and high relative humidity (Romero 2009). This may allow *R. iberica* to be active throughout the year and also have an extended breeding period with continuous gametogenic activity. Yet, phenology studies in lower altitude populations in Serra da Estrela would allow a better understanding of the biology of these underground residents.

Adults were found frequently in mating attempts and in amplexus. Occasionally, more than one male was seen trying to mate with a female, which has also been re-

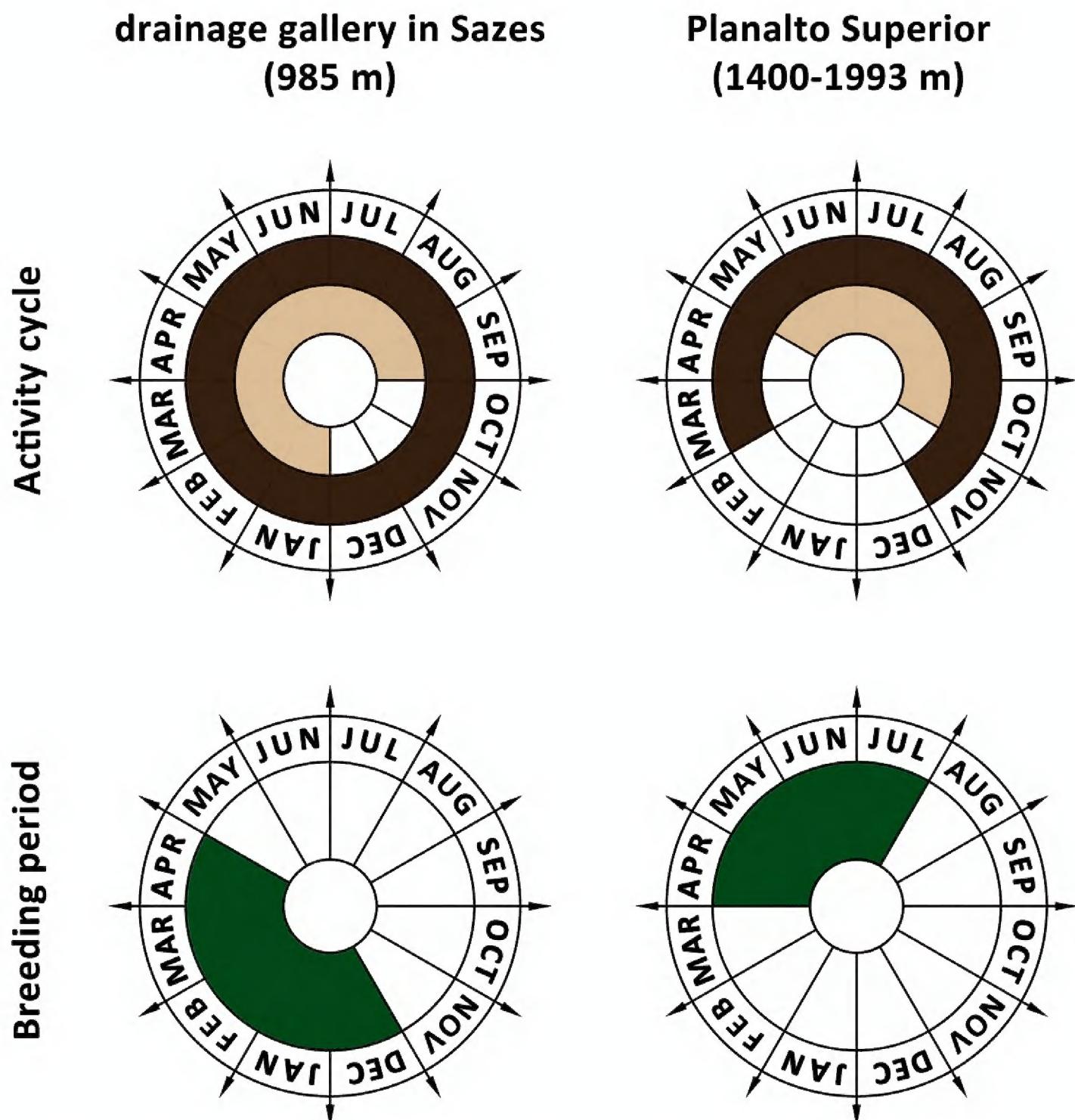


Figure 2. Polar coordinates representing the activity cycle and breeding period of *Rana iberica* in two different sites (inside the drainage gallery in Sazes and the whole area of Planalto Superior) in Serra da Estrela; Portugal. Dark brown areas: post-metamorphic phase; beige areas: larval phase; green areas: adults in breeding activity.

ported by Ayres (2008), which seems to be more common than previously thought. Amplexus was not limited to nighttime. Mating occurred primarily in water (Fig. 3D), but also was observed on land (Fig. 3E) on the margins of the stream and on rocks mainly on the floor of the gallery.

Clumps of eggs with a gelatinous coat were generally laid and stuck to submerged rocks in the stream (except for one case: see Fig 5A and description below) and recently hatched tadpoles could be observed stationary above the egg mass for about two weeks (Fig. 4 A–B). Tadpoles were pale grey in coloration and became darker during the second week (Fig. 4C; Ayres 2009).

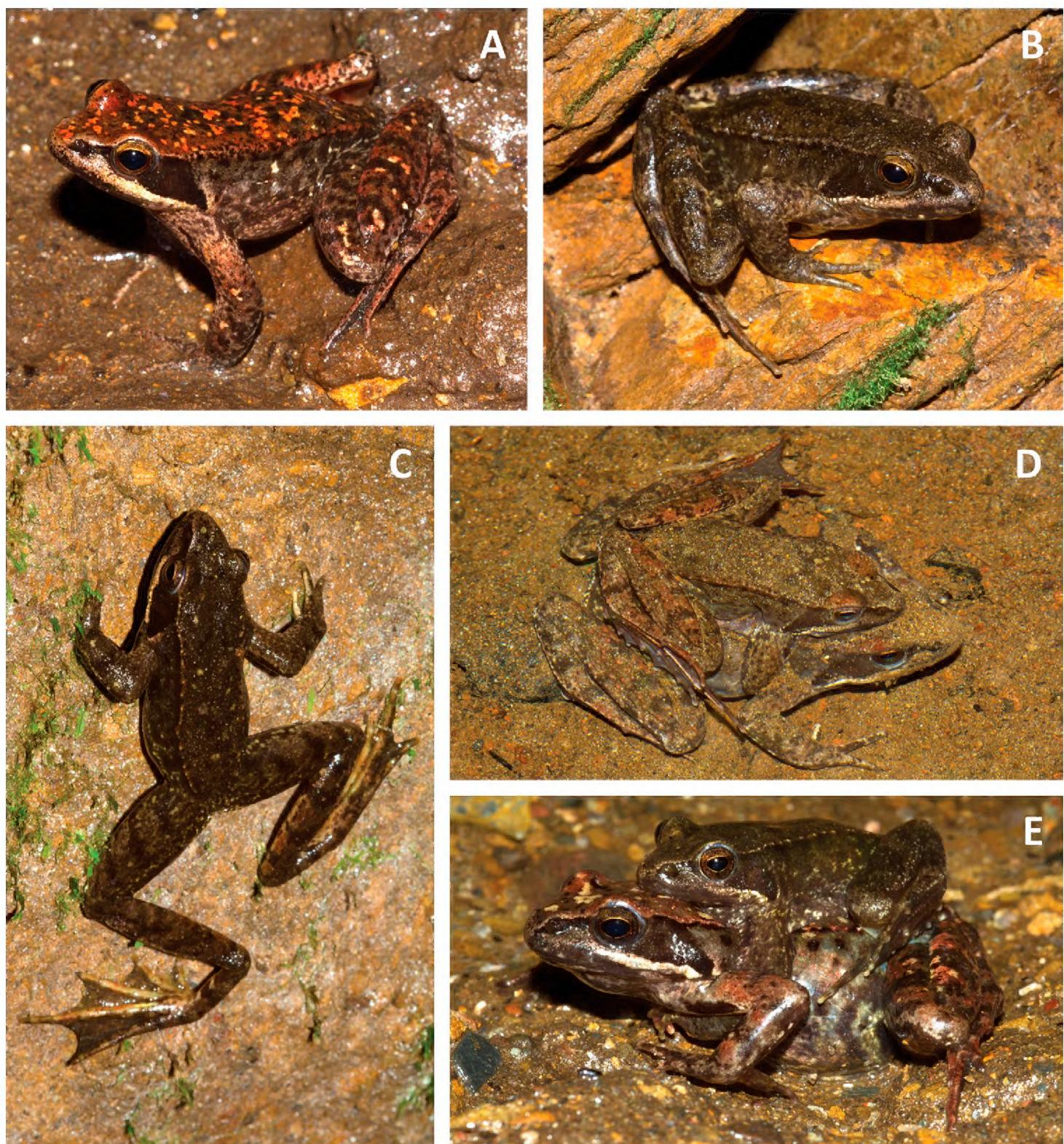


Figure 3. Adult individuals of *Rana iberica* found inhabiting a drainage gallery in Serra da Estrela, Portugal: **A** male with typical lichen-shaped pattern on the back **B** female hidden in a crevice of the gallery **C** male climbing up the wall **D** couple in axillary amplexus in water **E** axillary amplexus out of the water. Photos by Rosa GM.

Facultative oophagy

Oophagy was observed in this subterranean population of *R. iberica*. A clutch of eggs was probably laid in water, but got stranded when the water level presumably dropped. When we first noticed, the mass was already > 90% above the water surface (Fig. 5A), preventing normal development of the eggs. This situation turned the egg mass into a source of nutrients for free swimming tadpoles (at least three weeks old) from other clutches. Several tadpoles were observed feeding on the eggs

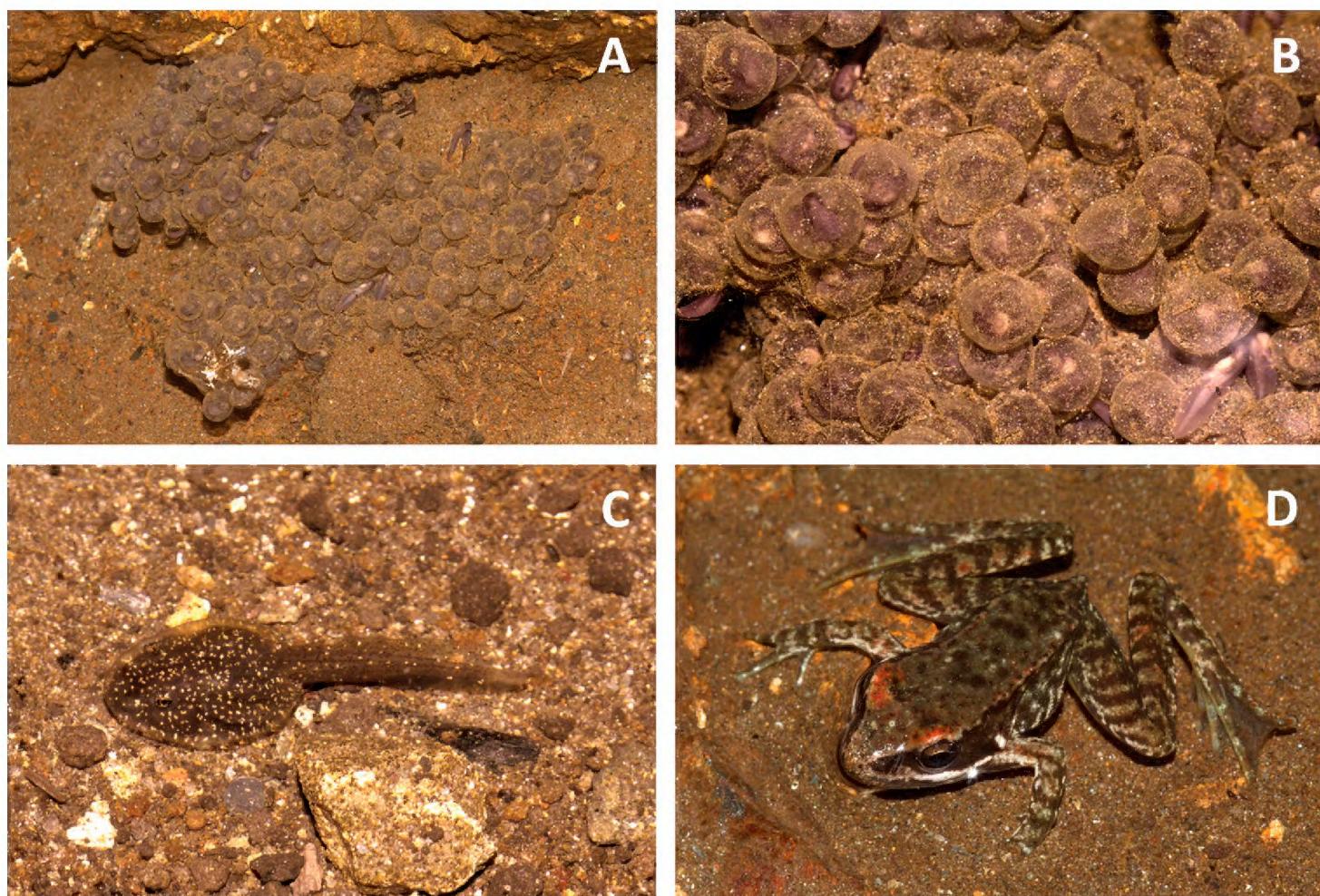


Figure 4. Egg and early life stages of *Rana iberica* inhabiting a drainage gallery in Serra da Estrela, Portugal: **A** egg mass stuck to underwater rock (28 January, 2012) **B** eggs' detail with new born tadpoles (one day old) **C** tadpole with dark pigmented colouration (Gosner stage 25; 11 March, 2012) **D** recently post-metamorphic individual (31 May, 2012). Photos by Rosa GM.

(Fig. 5B–E), a behaviour also reported for the first time for this species. Conspecific oophagy is a form of cannibalism (Miaud 1993) often associated with the intentional production of trophic eggs to feed young (e.g. Brust 1993, Gibson and Buley 2004), however this seems not to be the case. Nevertheless, facultative oophagy may be a more common and widespread habit of this species but also across other tadpoles of the same genus, that would take place when egg clutches are lost and larvae don't hatch.

Predation by fire salamander larvae

The gallery is also inhabited by other amphibians, including female *Salamandra salamandra gallaica* that use the underground spring to give birth to small larvae. During one visit to the gallery on the 29 April 2012, two predation events were recorded. Two *S. s. gallaica* larvae (post stage 46; Harrison 1969) were observed ingesting *R. iberica* tadpoles (stage between 26–30; Gosner 1960). In the first case, we observed a salamander larva slowing approach a tadpole and when the larva was less than a centimetre from the tadpole's flank (Fig. 6A), it lunged rapidly and seized the tadpole by the tail. A second salamander larva was found grasping a tadpole in its mouth with the hind

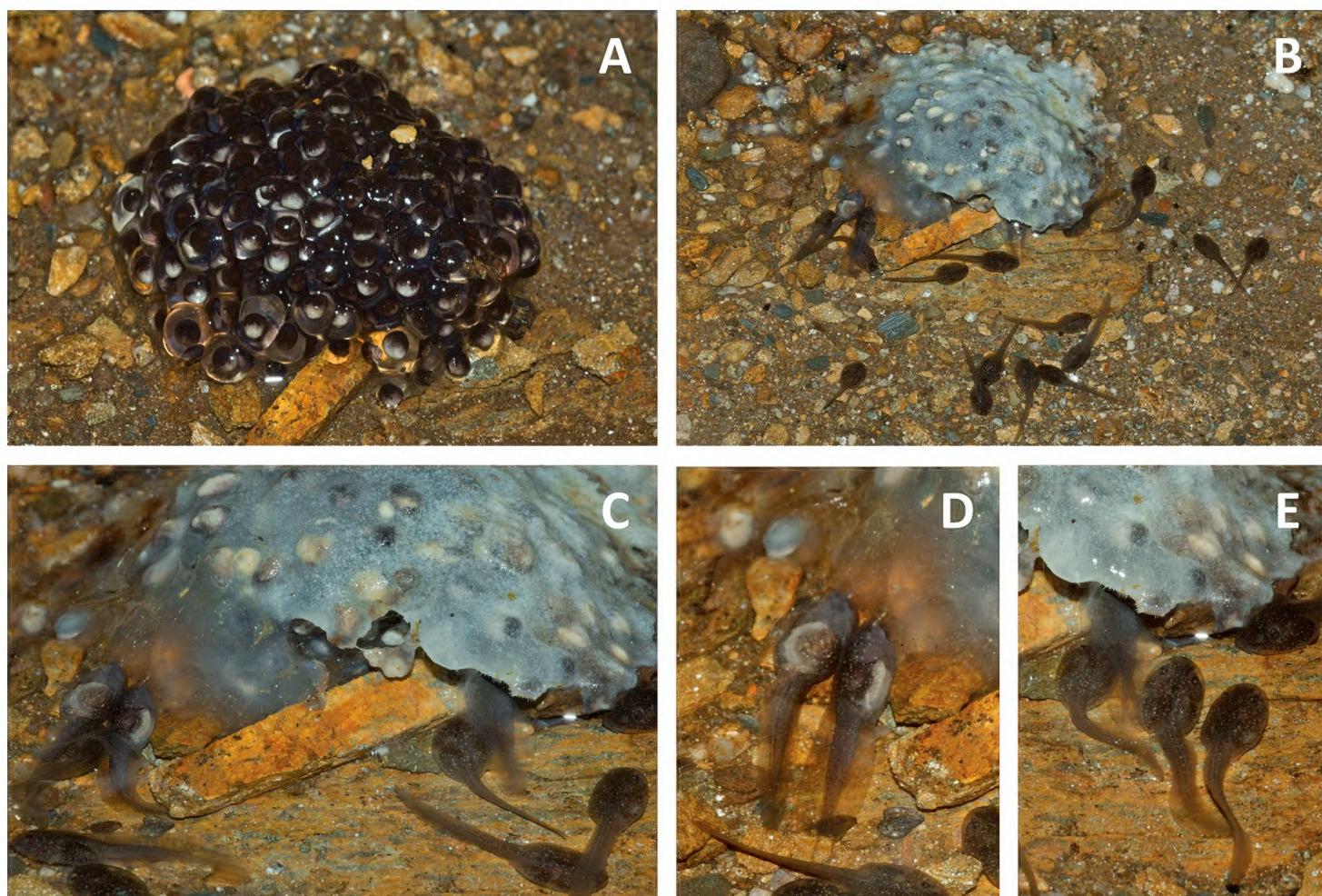


Figure 5. *Rana iberica* tadpoles feeding on lost clutch: **A** fresh egg mass of *Rana iberica* laid (mostly) above water surface (11 March, 2012) **B** group of tadpoles feeding on the dead eggs (29 April, 2012) **C, D** and **E** close ups of tadpole feasting on the eggs. Photos by Rosa GM.

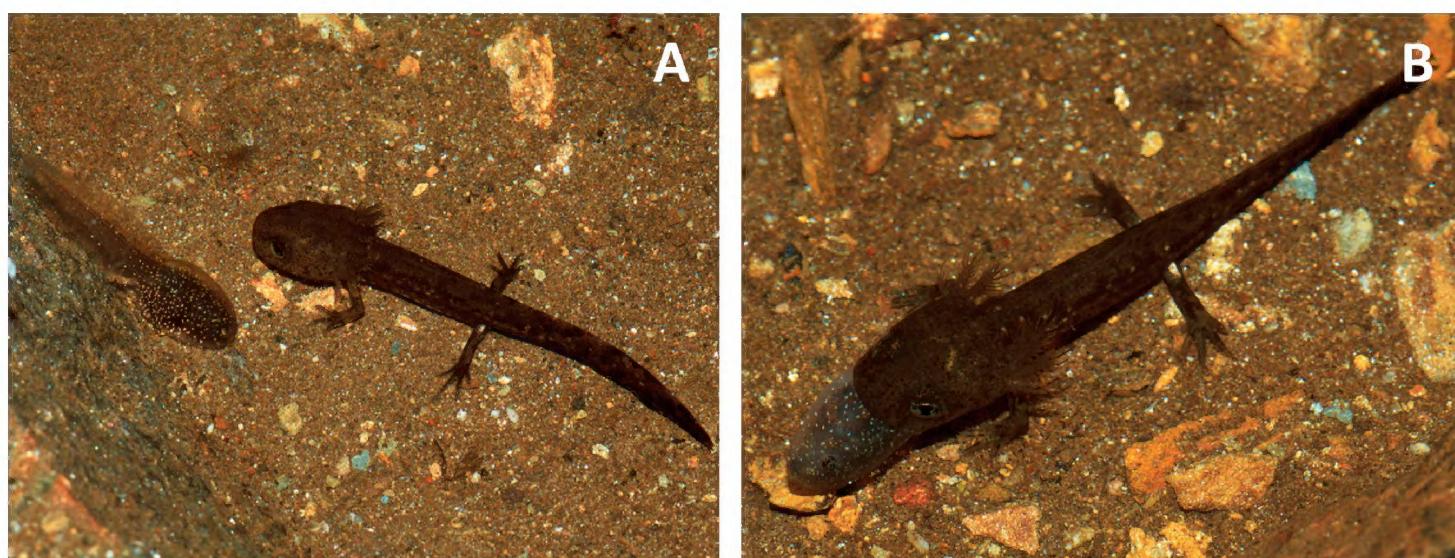


Figure 6. *Salamandra salamandra gallaica* larvae predation upon *Rana iberica* tadpole on the 29 April (2012): **A** individual of salamander approaching tadpole instants before seizing it **B** salamander larval ingesting tadpole. Photos by Rosa GM.

part of the body (including the whole tail) already ingested (Fig. 6B) later that same day. These observations represent the first report of predation by Fire salamanders on Iberian brown frog. We believe that this is not a behaviour exclusive to subterranean habitats and that predation might occur more frequently in surface habitats shared by larvae of both species.

Final considerations

Anurans from the genus *Rana* appear to be the most common anuran visitors of caves and similar subterranean habitats, both in the Nearctic and the Palearctic regions (Resetarits 1986, Resetarits and Aldridge 1988, Bressi and Dolce 1999, Fenolio et al. 2005, Manenti 2008). Our observations represent the first record of *Rana iberica* reproduction in cave-like habitat and the fourth report of an anuran utilizing a cave or subterranean habitat during all stages of its life cycle for the Palearctic ecozone. It is unknown whether *R. iberica* exploits subterranean habitats elsewhere or why this particular population does so. Nearby breeding sites may have disappeared or experienced anthropogenic disturbance. For example, one of the artificial tanks was emptied during the summer of 2011 by the forestry services to be repaired, and frogs may have searched for alternative sites to lay their eggs, such as springs in drainage galleries.

Additionally, few other questions have been raised: is the presence of the species in the hypogean habitat temporal and accidental, or permanent? Does this apply to the entire population or only a part of it? Is the hypogean population in contact with the nearby epigean ones, and if so, where and when? A mark-recapture study (possibly by taking advantage of the characteristic dorsal pigment pattern of adult frogs) might help to shed some light on these questions, providing a better insight into the dynamics of this interesting population.

The Mediterranean region has experienced dramatic shifts in climate in the past (Luterbacher et al. 2006). This region has been identified as a hotspot of biodiversity but may face increased aridity in the future based on climate change projections (Giorgi 2006, Gao and Giorgi 2008) and intensive fire activity (Azul et al. 2010). The Iberian amphibian community may face huge ecological challenges (Bosch et al. 2007, Leadley et al. 2010, Carvalho et al. 2011) and artificial subterranean habitats, such as drainage galleries, may act as refugia for the survival of some populations.

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